Summary of the Invention

An object of the present invention is to provide a method for detecting molecules expressing a selected epitope in a sample. In this method, an epitope anchor specific for a selected epitope is immobilized to a selected surface. The epitope anchor may comprise a single chain Fv fragment, a CDR, an antibody, or other ligand peptide or chemical or pharmaceutical that interacts with a selected epitope. The surface is then contacted with a sample suspected of containing molecules which express the selected epitope so that the molecules bind to the immobilized epitope anchor. An epitope detector comprising a single chain Fv for the selected epitope or a constrained epitope specific CDR attached to an oligonucleotide, preferably a double-stranded cDNA, is then used to detect any bound molecules. Alternatively, the method of the present invention can be performed without an epitope anchor. In this embodiment, the epitope detector is employed to define molecules bound directly to a surface.

In a preferred embodiment of this method, detection is performed via readout using a standard fluorimetric device of a fluorescence derived numerical value. More specifically, in this embodiment, following amplification of the oligonucleotide of the epitope detector, a fluorescent dye is incorporated into the nucleic acid sequence in a linear manner. Upon excitation at a selected wavelength, the dye yields a quanta of fluorescence signals directly proportional to the mass of sample loaded.

With a mixture of epitopes detectors comprising either monoclonal antibodies for selected epitopes, single chain Fvs for selected epitopes or constrained epitope specific CDRs, conjugated to cDNAs of different lengths, the method of the present invention can also be used to profile proteins in a cell lysate.

In a preferred embodiment, the oligonucleotide is double-stranded and comprises a T7 promoter driven cDNA sequence so that it can be amplified using T7 RNA polymerase. In this embodiment, double-stranded cDNA is synthesized for use as a template for T7 RNA polymerase transcription. T7 RNA polymerase requires its promoter site to be double-stranded.

In one embodiment, the site on the Fv or CDR to which the oligonucleotides are attached comprises a series of residues which allow the attachment of linkers consisting of chemicals such as heterodimeric coupling reagents or other linkers. These residues provide a uniform binding site for the linker attachment. The linkers attach to this site and also link oligonucleotides to the Fv or CDR. Oligonucleotides may be unmodified or modified. For example, the presence of the amplified oligonucleotide can be enhanced by incorporating a beacon or fluorescent labeled oligonucleotide into the mixture allowing for rapid semi quantitative assessment of the epitope expressing molecules (Ton et al. Chemistry 2000 6:1107-1111; Leone et al. Nucleic Acids Res. 1998 26(9):2150-2155).

In another embodiment, the oligonucleotide of the epitope detector is coupled to biotin and the monoclonal antibody, single chain Fv or constrained epitope specific CDR is coupled to streptavidin and attachment of the oligonucleotide to the monoclonal antibody, single chain Fv or constrained epitope specific CDR occurs via complexing of the biotin to the streptavidin.

Bound epitope detectors may be quantified by methods such as amplification by conventional PCR or aRNA techniques. If the detection method used is immuno aRNA, double-stranded cDNA are preferably used in the epitope detector. In this embodiment, aRNA is transcribed on the solid support using a polymerase which recognizes a specific promoter such as T7 RNA polymerase, T3 RNA polymerase, SP6